



TITLE:

Lineage isolation maintained by natural selection despite ongoing gene flow in Japanese wild radish( Digest\_要約 )

AUTHOR(S):

Han, Qingxiang

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CITATION:

Han, Qingxiang. Lineage isolation maintained by natural selection despite ongoing gene flow in Japanese wild radish. 京都大学, 2017, 博士(人間・環境学)

ISSUE DATE:

2017-03-23

URL:

<https://doi.org/10.14989/doctor.k20459>

RIGHT:

学位規則第9条第2項により要約公開

## 論文要約

論文題目 Lineage isolation maintained by natural selection despite ongoing gene flow in Japanese wild radish (遺伝子流動存在下で自然選択によって維持されている日本のハマダイコンの系統隔離)

申請者 韓 慶香

### 論文要約

This work focused on the role of gene flow and natural selection in the process of population divergence in wild radish, a typical coastal plant, and the mechanisms by which flowering plants respond to different environments. The goal of this research was to provide new insight into the processes of intraspecific differentiation and adaptive evolution.

Wild radish, *Raphanus sativus* var. *raphanistroides* Makino, is widely distributed in sandy coastal environments in East Asia. This species is characterized by seawater-dispersed seeds surrounded by spongy tissue and/or air-filled cavities in the pericarp, which allow the seeds to disperse over long distances. Because of the breadth of its dispersal, which facilitates frequent gene flow between populations, wild radish could be expected to lack geographical genetic variation. However, considering that wild radish is widely distributed around the entire Japanese coast and over large latitudinal ranges, and given that it occupies systematically varied habitats, it seems probable that divergent natural selection can generate ecological barriers, restricting gene flow and driving intraspecific differentiation. In-depth study of the phylogeography and demographics of wild radish is necessary to test these two opposing hypotheses. To obtain a better understanding of the adaptive evolution of wild radish, I investigated variations in flowering time and leaf morphology of wild radish plants from mainland Japan and the Ryukyu Islands. These phenotypic traits might reasonably be expected to show lineage-specific variations in response to selective forces imposed by divergent habitats. This work will aid in understanding the roles of gene flow and natural selection in the lineage isolation of plants with wide ranges, and how such plants adapt to differing environments.

In chapter 1, to investigate the phylogeographic structure of wild radish in Japan, I conducted multiple nuclear DNA microsatellite loci and chloroplast DNA haplotype analyses. Cluster analysis suggested the existence of differential genetic structures between the mainland Japan (northern) and Ryukyu Islands (southern) clusters. I detected a significant, strong pattern of isolation by distance, as well as significant evidence of a recent bottleneck. Chloroplast marker analysis showed that predominant haplotypes were shared among all populations, a phenomenon that might be most plausibly explained by the retention of ancestral haplotypes (*i.e.*, the coalescent effect). The results indicate that the Kuroshio Current has contributed to the sculpting of the phylogeographic structure by reducing effective seed flow between the mainland and Ryukyu Islands, which in turn contributed to genetic gaps between these populations. In addition, the Tokara Strait between Amamioshima

Island and Tanegashima Island may have created a geographic barrier that restricted gene flow between the Ryukyu and mainland populations. Finally, extant habitat disturbances (coastal erosion), migration patterns (linear expansion), and geographic characteristics (small islands and sea currents) have collectively influenced the expansion and historical population dynamics of wild radish. This work is the first to record the robust phylogeographic structure of wild radish populations from the Ryukyu Islands and mainland Japan, and it provides insight into geographical genetic variation in this species on a local scale.

In chapter 2, I discuss my survey of nucleotide polymorphisms over multiple nuclear loci utilizing the isolation-with-migration (IM) model to estimate parameter values to identify demographic changes and key factors that have shaped the population history of Japanese wild radish. In addition, I investigated the flowering times of individuals from different wild radish lineages, with and without cold exposure, to assess their respective vernalization responses. Coalescent simulations suggested that the southern and northern lineages of wild radish began to diverge ~18,000 years ago, during the Last Glacial Maximum (LGM) period. Gene flow was considerably higher from southern to northern groups than in the opposite direction, indicating the effective dispersal of viable seeds via the northward Kuroshio Current. The south-north lineage divergence in wild radish might have been triggered by a directional change in the sea current during the LGM period, despite gene flow resulting from the high dispersability and longevity of seeds. Further cultivation experiments indicated that cold exposure was not required to initiate flowering in the southern group, but could advance the date of flowering, suggesting that vernalization is facultative in the southern group. In contrast, the northern group was either unable to flower or flowered later without prior cold exposure. Thus, different vernalization requirements resulting in different flowering times might lead to partial or complete seasonal isolation and favor prezygotic reproductive isolation between lineages, and ultimately enhance and maintain intraspecific differentiation of wild radish. This study provides new insight into the maintenance of lineage differentiation with ongoing gene flow in coastal plants.

In chapter 3, I describe the analysis of variations in flowering time in response to cold treatment and day length, and the interaction between vernalization and photoperiod pathways, in wild radish collected on Hokkaido Island (northern lineage) and Okinawa Island (southern lineage), which have considerably different environments. The objectives of this chapter were: 1) to identify the gene network controlling flowering in wild radish with different vernalization requirements (obligate and facultative), and 2) to identify climatic signals that might act as natural selective forces shaping the genetic variation determining flowering initiation. Gene expression analysis showed an antagonistic effect between *RsFLC-A* and *RsCOL5-A*, controlling flowering time in northern and southern accessions, respectively. Notably, in non-vernalized southern accessions, flowering time could be promoted by the active expression of *RsCOL5-A*, even when transcript levels of *RsFLC-A* were high. These results collectively indicated that northern lineages exhibited a higher sensitivity to prolonged cold exposure, whereas southern accessions were more dependent on the photoperiod pathway. Over generations, these differences eventually confer an optimal flowering time in natural populations in response to contrasting climate cues. This work is of

great importance in understanding how plants alter their flowering times in response to different environments.

In chapter 4, I investigated geographic variation in leaf trichomes in wild radish populations across Japan, covering the Ryukyu Islands and the mainland, to gain a better understanding of how morphological variation can be moulded by natural selection. I also sought to illuminate the selective pressures involved in the evolution and maintenance of leaf trichome polymorphisms. I found that leaf trichome density in wild radish was highly variable across the Japanese archipelago, with plants from southern insular populations tending to exhibit glabrous leaves, while the leaves of plants from northern mainland Japan were completely covered by dense trichomes. This trichome variation in Japanese populations of wild radish may be the result of various external stressors, including frost damage and predation by herbivores. This study contributes to the understanding of variation in phenotypic traits of wild radish under divergent natural selection.

In summary, this work demonstrates that northern and southern lineages of wild radish diverged approximately 18,000 years ago, despite the homogenizing force of gene flow caused by a combination of frequent long-distance seed dispersal via ocean currents and the high longevity of seeds of this plant. Furthermore, northern (mainland Japan) and southern (Ryukyu Islands) lineages exhibited different vernalization requirements, with the process being obligate in northern accessions and facultative for southern lines. Additionally, Hokkaido accessions (northern lineage) exhibited a higher sensitivity to prolonged cold exposure, whereas Okinawa accessions (southern lineage) relied predominantly on the photoperiod pathway, differences that may have arisen in response to climate cues. In addition, geographic variation in the density of leaf trichomes in wild radish populations across Japan, including the Ryukyu Islands and mainland, reflect divergent natural selection between northern and southern lineages. Overall, these results indicate that differential phenotypic traits of vernalization requirements, preferential floral pathway, and leaf morphology have probably acted as divergent selective forces, contributing to partial or complete seasonal isolation and favoring prezygotic reproductive isolation between lineages, which have ultimately driven and maintained divergence between northern and southern lineages of wild radish despite active gene flow between these populations. This work increases our understanding of how lineage isolation in flowering coastal plants is maintained by divergent natural selection generated by differing environments in the face of ongoing gene flow. This work also offers a new and fascinating story about wild radish, which is helpful to understand the mechanisms of adaptive evolution, particularly in the case of coastal plants, on a local scale.